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Photoautotrophy in Gymnodinium breve Davis

Abstract. Pure cultures of the Florida "red-tide" flagellate required light and carbon dioxide for growth. Multiplication in darkness was not supported by any of a number of organic compounds and mixtures. The ecological importance of micronutrients is suggested.

The catastrophic mortalities of marine animals associated with dense populations of the dinoflagellate, *Gymnodinium breve* Davis, have occasioned considerable interest in the biological requirements of this organism (*1*). Several environmental factors have been suggested as agents contributing to "red-tide" outbreaks which recur along the Florida Gulf coast. Such phenomena have been more closely associated with extended periods of heavy rain than with any other condition (*2*). In view of the prevalence of organic soils in this locality (*3*), and the fact that aqueous extracts of organic soil promoted growth in the first successful cultures of *G. breve*, it seemed that river discharge, representing soil drainage, might be "instrumental in initiating" blooms of this organism (*4*). Wilson and Collier also suggest that decaying red-tide-killed fish "imparted nutrients that helped to perpetuate the bloom" (*4*). In the light of references to the widespread occurrence of heterotrophy among dinoflagellates (*5*), it seemed appropriate to determine whether the organic content of Florida river waters or coastal fishes might provide a direct source of energy for multiplication in *G. breve*. Accordingly, my report represents an attempt to define the heterotrophic capacities of this organism.

Axenic, 10-ml cultures of the flagellate were prepared in screw-capped tubes, using about 200 motile cells for inoculum, and a virtually inorganic, artificial sea water medium (*6*). Since the basic medium permits neither multiplication nor survival of *G. breve* in darkness, it was assumed that the degree to which added substances could serve as energy sources would be related to their abilities to support multiplication in the dark. The extreme sensitivity of this organism renders the incidence and rate of multiplication somewhat unpredictable, even in replicate cultures. This type of variation in results was avoided by starting all cultures with the basic medium only and illuminating them (500 ft-ca) until successes and failures were obvious at 10 × magnification. Cultures showing comparable growth (at least a tenfold population increase within 4 weeks) were then selected for use in this work. Aseptic additions of the organic compounds were made to all except control tubes, and the cultures were placed in darkness. An identical series of enriched and unenriched cultures was illuminated as above. All cultures were maintained at 25° ± 1°C. Estimates of population growth and survival were made microscopically.

One of the Florida soil extracts tested (prepared as described in (*4*) from soil obtained in the Fort Myers area) had also been used in natural Florida sea water media, in which it aided multiplication of *G. breve* in illuminated cultures. Each soil extract was tested at concentrations of 10 and 100 ml/liter. The fish extracts represented a filtered, 10-percent composite homogenate of muscle tissue from anchovy (*Anchoa mitchilli*), sand seatrout (*Cynoscion arenarius*), spot (*Leiostomus xanthurus*), Atlantic croaker (*Micropogon undulatus*), and striped mullet (*Mugil cephalus*), all common inhabitants of Florida west coast waters. This type of extract (either fresh or aged 3 days with uninhibited bacterial growth) was tested at a concentration of 5 ml/liter. The other organic substances were tested at several concentrations (0.1, 1.0, and 10.0 mg/liter) chosen to include and exceed the usual range of carbohydrate and total tyrosine concentrations found in Florida coastal water (*7*).

None of the organic compounds or mixtures (Table 1) supported multiplication or permitted extended survival in darkness. The longest survival was in the valeric acid cultures, which showed a 90-percent population reduction in 2½ weeks. No population increase was observed, however, in six replicate newly inoculated cultures containing valeric acid. All other cultures, both control and enriched, lost at least 90 percent of their initial populations in 1 to 2 weeks. Most of the identically enriched illuminated cultures did not decrease in population size during the same period, thus indicating no inhibitory effect for most of the compounds tested. The four exceptions contained 10 mg of linoleic acid, sodium oleate, sodium stearate, or kinetin per liter. Attempts to facilitate gas exchange by using very loosely packed sterile cotton plugs in place of the regular screw caps failed to extend survival without light. Attenuation of light over a period of several days, when introducing experimental cultures to darkness, also failed

Table 1. Organic substances tested as potential energy sources for *Gymnodinium breve*.

Substance	Substance
Carbohydrates and related compounds	*Lipids (Contd.)*
Arabinose	Butyrate
Ribose	Valerate
Xylose	Palmitate
Galactose	Stearate
Glucose	Oleate
Mannose	Linoleate
Fructose	
Sorbose	*Nitrogen compounds*
Rhamnose	Urea
Cellobiose	Alanine
Lactose	Aspartate
Maltose	Glutamate
Melibiose	Serine
Sucrose	Asparagine
Trehalose	Creatine
Melezitose	Adenine
Raffinose	Gelatin
α-methyl glucoside	
Glycogen	*Growth factors*
Inulin	Ascorbic acid
Pectin	Dehydroascorbic acid
Soluble starch	Biotin
	Cobalamin
Alcohols	Thiamine
Ethanol	Kinetin
Butanol	
Dulcitol	*Miscellaneous compounds and mixtures*
Mannitol	Beef blood serum
Sorbitol	Casamino acids
Ethylene glycol	Casein hydrolyzate
Glycerol	Coconut milk
Glycolytic and citric acid cycle constituents	Egg yolk
Glucose-1-phosphate	Fish extract
Fructose-6-phosphate	Liver concentrate
Phosphoglycerate	Malt extract
ATP	Mangrove extract
Citrate	Milk
Pyruvate	Milk protein hydrozyate
Isocitrate	Oxalate
α-ketoglutarate	Peptone
Succinate	Peptone-iron
Fumarate	Thioglycollate
Malate	Tartrate
Oxaloacetate	Tryptone
Lactate	Florida soil extracts (Ft. Myers) (St. Petersburg)
Lipids	Texas soil extract (San Antonio)
Formate	Yeast extract
Acetate	
Propionate	

to alter these results. Furthermore, removal of CO_2 from the medium by suspending 0.2 ml of 10 percent KOH in the gas phase of established cultures and sealing the tubes with paraffin prevented multiplication or survival of *G. breve* in illuminated cultures. (Similarly sealed cultures with 0.2 ml of distilled water suspended in the gas phase showed no change in population.)

One must conclude that this dinoflagellate is primarily photoautotrophic, requiring light and CO_2 for growth and survival. The present findings parallel those of Barker and of Provasoli and co-workers, who did not find organic metabolic substrates capable of supporting growth of photosynthetic dinoflagellates in darkness (*9*). The growth-stimulating effects of thiamine, biotin, and cobalamin in illuminated cultures (*8*) represent the only known deviation from strict photoautotrophy in *G. breve*. Provasoli also noted the importance of B vitamins to the multiplication of such organisms.

A compound capable of supporting growth in darkness can be overlooked in this type of study, as Lewin has pointed out (*10*). However, the present findings suggest that heterotrophy is not an important consideration in the development of the astronomical *G. breve* populations involved in Florida red-tide outbreaks.

If Florida west coast rivers do not provide direct energy sources for multiplication of *G. breve*, the organism's vitamin, trace-metal, and chelator requirements assume added ecological significance as factors potentially limiting population growth. Similar indirect evidence may be seen in extensive field data (*11*), which suggest that phosphate and nitrate are not usually growth restricting to this form in nature.

Vitamins, trace metals, and chelators may be introduced by river waters into coastal environments. Provasoli (*12*) has pointed out broad ecological implications: "It is well known that the waters near estuaries, inlets, and inshore are fertile areas; this fertility should now be considered not only in terms of phosphates and nitrogenous compounds but also of vitamins, trace metals, and chelating agents." The natural fluctuations in levels of such substances may also be a fruitful area for future study of *G. breve* and red-tide ecology.

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References and Notes

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